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(54) Title: AGONISTS OR ANTAGONISTS FOR HAEMOPOIETIC GROWTH FACTORS

(57) Abstract

An agonist or antagonist of a haemopoietic growth factor that is capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding haemopoietic growth factor receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property. The agonist or antagonist properties may be to a member of the cytokine receptor family where the member is selected from any one of a group acting as a receptor for any one or more of IL–2, IL–3, IL–4, IL–5, IL–6, IL–7, IL–9, IL–11, IL–13, IL–15, granulocyte–macrophage colony stimulating factor (GM–CSF), growth hormone (GH), prolactin (PRL), granulocyte colony stimulating factor (G–CSF), erythropoietin (EPO), thrombopoietin (TPO), leukaemia inhibitory factor (LIF), oncostatin–M (OSM), ciliary neurotrophic factor (CNTF), the p40 subunit of IL–12, leptin and newly discovered members of the cytokine receptor family.

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AGONISTS OR ANTAGONISTS FOR HAEMOPOIETIC GROWTH FACTORS

FIELD OF THE INVENTION

This invention relates to agonists and antagonists to certain haemopoietic growth factors and to methods of isolating the agonists and antagonists and to therapeutic use of the agonists or antagonists.

INTRODUCTION

Granulocyte-macrophage colony stimulating factor (GM-CSF), Interleukin-3 (IL-3) and Interleukin-5 (IL-5) are cytokines involved in hemopoiesis and inflammation and all three stimulate eosinophil production, function and survival and therefore have the ability to influence inflammatory diseases such as asthma, atopic dermatitis and allergic rhinitis where the eosinophil plays a major effector role. In addition, GM-CSF and IL-3 also stimulate the proliferation, differentiation and functional activity of a wider variety of haemopoieteic cells including neutrophils, monocytes and early progenitor cells.

GM-CSF, IL-3 and IL-5 have overlapping, pleiotropic effects on haemopoietic cells and these effects include mitogenesis, protection from apoptosis, differentiation and functional activation. The overlapping activities observed with these cytokines is explained by the fact that the high affinity receptors for human GM-CSF, IL-3 and IL-5 share a common β-subunit ($h\beta_c$). These three receptor complexes (GMR, IL3R and IL5R), which are discussed in detail by Miyajima *et al.*(1), are each comprised of unique ligand-specific subunits (GMRα, IL3Rα or IL5Rα) and a shared subunit ($h\beta_c$), which appears to be the primary signalling subunit (2). While it is clear that $h\beta_c$ is required for signalling it is still uncertain whether the α subunits have a direct signalling role. Although their cytoplasmic segments are clearly required for the normal function of these receptors (4-7), as yet no signalling molecules have been detected that associate with α subunits, and it appears that under some circumstances, β subunit dimerisation is sufficient for signalling (8-10). The stoichiometry of the active receptor complex has not been defined. Whilst signalling via ββ dimers suggests a higher order complex, there is no definitive published evidence that rules out the simple model that an $\alpha_1\beta_1$ dimer can deliver intracellular signals - in fact this is consistent with results obtained using fos- and jun- leucine-zipper receptor subunit chimeras (11).

The receptor subunits for GM-CSF, IL-3 and IL-5 are all members of the cytokine receptor (CR) superfamily (12, 13) which is characterised by a two hundred amino acid, extracellular, CR module (CRM). Each CRM comprises two fibronectin-like β

barrel structures (CR domains, CRD (14)); the seven β-strands in the membrane-distal and -proximal CRDs are designated A to G and A` to G`, respectively. Within the membrane-distal CRD are two pairs of disulfide-linked cysteine residues, while the membrane-proximal CRD of nearly all CRs contains a motif with the consensus Trp-Ser-Xaa-Trp-Ser (where Xaa is any amino acid) - referred to as the "WSXWS box". In addition to the GM-CSF, IL-3 and IL-5 receptor subunits other members of this family include the receptor subunits for growth hormone (GH), prolactin (PRL), erythropoietin (Epo), thrombopoietin (Tpo), Interleukin-2 (IL-2), IL-4, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, Granulocyte-colony stimulating factor (G-CSF), leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), the p40 subunit of IL-12 and most recently the leptin receptor (15).

A number of activating mutations in $h\beta_c$ have now been described. These have been informative with respect to the activation of the GM-CSF receptor and are summarised in a recent review (16). All of the mutants were isolated on the basis of their ability to confer factor-independent growth on the normally factor-dependent murine myeloid cell line, FDC-P1. A common feature of a large group of these mutants is that the mutations affect the membrane-proximal CRM of the extracellular portion of $h\beta_c$ (CRD3 and CRD4). *In vitro*, these mutants confer factor-independence only on myeloid (granulocyte-macrophage) cells (17), a restriction explained by the recent finding that in mouse cell lines the CRD4 mutants require and interact with the (murine) GM-CSF receptor α-subunit (mGMRα)(18). These mutants also have activity in a subset of human haemopoietic cell lines suggesting that cell type-specific interactions are also important for activity in human cells (18). Interestingly, most of the extracellular activating amino acid substitutions affect hydrophobic residues that are in β-strands and several of these residues are highly conserved within the CR family (16,19) (WO 97/07125 to D'Andrea *et al*). We have proposed that CRD4 acts as a conformational switch and that normally, in the absence of ligand, it is constrained in an inactive conformation which cannot form a functional complex with the mGMRα (16). The extracellular mutations in CRD4 appear to act by inducing a conformational change that results in an association with a mGMRα subunit (18). We suggest that in the normal human receptor the active conformation of CRD4 is induced by ligand association allowing a functional association with hGMRα. This is consistent with the observation that there are crucial contacts between ligand and $h\beta_c$ within CRD4 (20-23).

The maintenance of the inactive conformation may be mediated through an interaction involving CRD3 and CRD4 (16,26). The structure of the inactive EPOR dimer

provides a model for such a structure (25). It has been shown previously that truncation of the extracellular region of hβc can result in activation; for example, a truncation equivalent to that which generated an oncogenic form of Tpo receptor (v-Mpl; ie which leaves only part of CRD4, see below) was shown to allow factor-independent proliferation of FDC-P1 cells (24). The critical event appears to be the loss of CRD3, since a truncation that retained all of CRD4, but removed CRDs 1, 2 and 3 was also activating, whereas removal of only CRDs 1 and 2 was insufficient to promote factor-independent proliferation (24) (WO 97/07125 to D'Andrea *et al*).

Activating mutations have also been characterised in other cytokine receptors. A constitutively active form of erythropoietin (EPO-R) containing an arginine to cysteine susbstitution in the membrane proximal CRD (at position 129) has been described. This R129C form of EPO-R forms disulphide linked homodimers in the absence of EPO suggesting that wild type EPO-R is activated by ligand induced homodimerisation (26). The introduction of further cysteine residues into the EPO-R membrane proximal domain also leads to disulphide linked homodimers that are constitutively active (27). *v-mpl* is a murine oncogene encoding an activated cytokine fusion protein v-MPL and is transduced by the murine myeloproliferative leukaemia virus (MPLV) (28). The *v-mpl* fusion was generated from the partially deleted and rearranged *env* gene fused with cellular sequences from the *c-mpl* proto-oncogene encoding the thrombopoietin (TPO) receptor (29).

Whilst Haemopoietic Growth Factors (HGF) have proved therapeutically useful, they are large proteins that have to be administered parenterally. The possibility that small molecules might replace these has been an attractive concept, but small molecules based on ligand are likely to be too complicated as a result of having to bind to the two (or more) receptor chains. Small molecules that sterically alter the receptor or induce an active conformation (in this case β_c) therefore could provide a similar spectrum of activity as ligand and be orally available. This advance would make therapy easier and possibly cheaper. The fact that these molecules would need to interact with receptor extracellularly would potentially simplify their design as they would not need to cross the cell membrane for function.

This work with $h\beta_c$ activating mutants has implications for the development of small molecule CR agonists. We predict that CRD3 and CRD4 of $h\beta_c$, or the equivalent domains of other CRs, would be ideal targets for isolating functional antibodies or small molecules. A small molecule that binds to a single binding site in one of these extracellular domains could induce receptor association if it was capable of affecting this

inhibitory interaction. It is important to stress that molecules of this type would be acting in a novel manner, by binding to a single CR subunit rather than by directly mediating receptor association as is the case for the erythropoietin and thrombopoietin peptide mimetics described recently (30-32). These peptides require contact with both subunits for function and thus it is unlikely that these molecules will be able to be reduced greatly in size and still retain function.

SUMMARY OF THE INVENTION

The present inventors have found a small deletion in CRD3 (Δ G254, A255) which is predicted to shorten the A-B loop and which confers constitutive activation. Additionally the inventors have data regarding a mutation in the adjacent E-F loop of CRD3 (Δ A314, T315). The present invention is predicated on the proposal that these residues are involved in interactions that are critical in modulating receptor activity. The A-B loop and the E-F loop of CRD3 are therefore both key regulatory loops in $h\beta_c$ and conservation in receptor structure throughout the CR family suggests a similar role throughout the superfamily. It is possible that the B'-C' and F'-G' loops of CRD4 that are important in ligand binding, may be involved in interactions with the A-B loop and E-F loop in the inactive receptor and that perturbation of these interactions constitutes one mechanism of activation. This structure may take a form that is similar to that described for an inactive EPOR dimer (25).

Interaction of ligand with these key loops in CRD3 or CRD4 may perturb inhibitory interactions in the same way as truncation or small deletion. This would result in conversion of the inactive receptor structure to an active structure capable of intracellular signalling. In the alternative they may result in inhibitory reactions that antagonise the actions of the cytokines that usually bind the receptors concerned.

Thus in a first aspect the invention could be said to reside in an agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding haemopoietic growth factor receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property.

As is known in the art, the membrane proximal CRD of nearly all CRs contain a motif with a consensus WSXWS sequence. It is also known that the CRs contain conserved VRVR and WSXWS motifs which flank regions of hβc and other receptors that are

known to be important for binding (see Middleton *et al.* (40); Woodcock *et al.* (22); Rajotte *et al.* (41); Layton *et al.* (42); Horsten *et al.* (43); Kalai *et al.* (44)).

The agonist or antagonist may have agonist or antagonist properties to a member of the cytokine receptor family where the member is selected from any one of a group acting as a receptor for any one or more of the following cytokines IL-2, IL-4, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, growth hormone (GH), prolactin (PRL), granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), thrombopoietin (TPO), leukaemia inhibitory factor (LIF), oncostatin-M (OSM), cilairy neurotrophic factor (CNTF), the p40 subunit of IL-12 and leptin. Alternatively the agent may be an agonist or antagonist of any one of GM-CSF, IL-5 and IL-3 or of a newly discovered cytokine that resembles other cytokines in structure or is known to bind to a receptor that has a predicted structure resembling that of a cytokine receptor. Further, the agent may function through a predicted cytokine receptor for which ligand is not yet identified.

The agonist or antagonist may be selected from any one of a number of classes of compounds including antibodies, fragments of antibodies (Fab, Fv or peptide fragments), peptides, oligosaccharides, oligonucleotides, or other organic or inorganic compounds.

Preferably the antagonist or agonist may be capable of binding to a region selected from a group comprising the A-B loop and the E-F loop.

In one preferred form of the invention the antagonist or agonist binds to the A-B loop of CRD 3 or an analogous domain within the family of haemopoietic growth factors.

The predicted A-B loop of CRD3 may be bounded by a cysteine residue at position 250 and 260 and having a tyrosine residue at position 262 ($h\beta_c$ numbering). The sequence of the A-B loop in analogous domains of other cytokine receptors can be predicted using the sequences between these conserved cyteine residues. Thus in one preferred form of the invention the antagonist or agonist may bind any one or more of the sequences listed in Figure 2.

In another preferred form of the invention the antagonist or agonist binds to the E-F loop of CRD3 or an analogous domain within the family of haemopoietic growth factors.

The predicted E-F loop of CRD3 may be bounded by a conserved cysteine in the E strand at position 306 and by an alternating series of hydrophobic residues in the F strand (positions 316, 321 and 323; see Figure 6) Thus in one preferred form of the invention the antagonist or agonist may bind any one or more of the sequences listed in Figure 6.

As discussed it is expected that the A-B and E-F loops of CRD3 are involved in interactions with the B'-C' and/or F'-G' loops of CRD4. Thus the antagonist or agonist may interfere with the interaction of the A-B loop of CRD3 or an analogous region within the family of haemopoietic growth factors with the F'-G' loop of CRD4 or an analogous domain within the family of cytokine receptors. Alternatively, the antagonist or agonist may interfere with the interaction of the A-B loop of CRD3 or an analogous region within the family of cytokine receptors, with the B'-C' loop of CRD4 or an analogous domain within the family of cytokine receptors.

In another embodiment of the invention the antagonist or agonist may interfere with the interaction of the E-F loop of CRD3, or an analogous domain within the family of cytokine receptors with the F'-G' loop of CRD4 or an analogous region within the family of cytokine receptors. Alternatively, the antagonist or agonist may interfere with the interaction of the E-F loop of CRD3, or an analogous region within the family of cytokine receptors with the B'-C' loop of CRD4 or an analogous domain within the family of cytokine receptors.

In a second aspect the invention could be said to reside in a method for isolating an agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_C$ or analogous domain of a corresponding cytokine receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property, said method including the steps of contacting candidate agonists or antagonists with CRD3 or fragments thereof, assaying candidate agents for their capacity to bind CRD3 or fragment thereof and testing for agonist or antagonist properties.

The method may include the step of isolating a monoclonal antibody to CRD3 using methods that are well known to those skilled in the art (Zola *et al.* (45)).

The method may include fixing CRD3 or a fragment thereof to a substrate, contacting the fixed CRD3 or fragment with candidate antagonists or agonists, washing away material not bound to the fixed CRD3 or fragment, identifying the bound candidate

antagonists or agonists, and testing the bound candidate antagonists or agonists for antagonist or agonist properties.

Methods of producing libraries of candidate antagonists or agonists are well known to those skilled in the art, and may include preparation of diversity libraries, such as random combinatorial peptide or nonpeptide libraries, that can be screened for molecules that specifically bind to CRD3 or a fragment thereof. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), natural products libraries, and in vitro translation-based libraries.

Screening the libraries can be accomplished by any of a variety of commonly known methods such as those found in PCT Publication No. WO 94/18318, for example. In a specific embodiment, screening can be carried out by contacting the library members with CRD3 or a fragment thereof (or nuclei acid or derivative) immobilized on a solid phase and harvesting those library members that bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318.

The agonist or antagonist properties of candidate agonists or antagonists may be tested using methods known in the art. Such methods may include monitoring the growth of factor-dependent cell lines in the absence of growth factor and the presence of a candidate molecule. Alternative approaches may include a biochemical assay to detect signalling, which assays might include any one of the following: tyrosine phosphorylation status of the receptor, the levels of phosphorylation on ERK1 and ERK2 MAP kinases, JAK and STAT tyrosine phosphorylation, STAT DNA binding activity, and activation of reporter genes induced by specific signalling pathways.

In a third aspect the invention could be said to reside in a pharmaceutical composition, said composition including an agonist or antagonist as described or defined herein, and a suitable excipient.

Methods for preparing pharmaceutical compositions are well known in the art as reference can be made to Remington's Pharmaceutical Sciences, Mack Publishing Company, Eaton, Pa., USA and may also include one or more pharmaceutically acceptable carriers and/or diluents.

In a fourth aspect the invention could be said to reside in a method of treating a condition in a human or animal by administering an agonist or antagonist of a member of the cytokine receptor family as described or defined herein in a pharmaceutically acceptable form, in a suitable carrier and in a therapeutically effective dose.

Conditions that may be treated include those that are currently treated by GM-CSF, IL-3 and IL-5 as well as those in which other members of the family of haemopoietic growth factors are used in treatment.

The antagonists of the present invention may be useful *inter alia* in the treatment of myeloid and lymphocyte leukemias, tumors of non-haempoeitic origins and acute and chronic inflammation such as asthma, rheumatoid arthritis and atherosclerosis. These and other conditions are considered herein to result from or be facilitated by the aberrant effects of an endogenous HGF such as GM-CSF, IL-3 or IL-5.

The treatment may be preventative and reduce the risk of contracting the condition or it may be used to alleviate or obviate the condition. The administration of the therapeutic agent can be in any pharmaceutically acceptable form in a suitable carrier, and in a therapeutically acceptable dose.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Model of CRD3 and CRD4 of $h\beta_c$. A model showing the predicted $h\beta_c$ CRD3/4 structure based on structures of other cytokine receptors (33). The spheres indicate the α-carbon atoms of residues Glycine 254 and Alanine 255 in the A-B loop. The arrows indicate the B`-C` and F`-G` loops of CRD4 which have been shown to be important in ligand contact (see text and 20).

Figure 2. Alignment of A-B loop sequences of cytokine receptors. The sequences of the predicted A-B loop for $h\beta_c$ and other related CRs are shown. Conserved cysteine and tryptophan residues are boxed. A highly conserved acidic residue (D253 in $h\beta_c$) within the loop region is underlined. The residues deleted in the ΔGA mutant are boxed.

Figure 3. Proliferation of FDC-P1 cells expressing the ΔGA form of hβc. Infected FDC-P1 cells grown in the presence (black) or absence (white) of mGM-CSF. Proliferation assays were performed as described in Materials and Methods. FDC-P1 cells infected with a wild type $h\beta_c$ retrovirus, an hβcFIΔ retrovirus (34) or a ΔGA retrovirus are shown.

Figure 4. Proliferation of BaF-B03 cells infected with retroviruses encoding activated forms of hβc. (A) Proliferation of BaF-B03 cells infected with the ΔGA mutant, (B) Proliferation of infected BaF-B03 expressing the murine GM-CSF α-subunit, infected with the ΔGA mutant. Cells were grown in the presence (grey) or absence (black) of murine IL-3. Control cell populations infected with a wild type $h\beta_c$ retrovirus, or a retrovirus carrying the constitutive mutant V449E (16) are also shown.

Figure 5. Proliferation of CTL-EN cells in response to hIL-3. Proliferation of CTL-EN cells expressing hIL3Rα and $h\beta_c$ mutants. Cells were grown in the presence of the indicated concentrations of human IL-3. A control cell population expressing only hIL3Rα is also shown.

Figure 6. Alignment of E-F loop sequences of cytokine receptors. The sequences of the predicted E-F loop for $h\beta_c$ and other related CRs are shown. The alignment consists of the three known structures (Growth hormone receptor, GHR, EPOR and the IL-6 receptor β-chain, gp130). The β-strand structure is indicated below the alignment; E: conserved β-strand, e: β-strand observed in at least one structure. Also included are the sequences of thrombopoietin and the leptin receptors (TPOR and OBR). The conserved cysteine in strand E and the hydrophobic residues in strand F are boxed. The residues deleted in the DAT mutant are underlined. and

Figure 7 Proliferation of BaF-B03 cells infected with retroviruses encoding the ΔAT mutant of $h\beta_c$. (A) Proliferation of BaF-B03 cells infected with the ΔAT mutant (B) Proliferation of BaF-B03 cells expressing the murine GM-CSF α-subunit, infected with the ΔAT mutant. Cells were grown in the presence (grey) or absence (black) of murine IL-3. Control cell populations infected with the pRUFNeo retrovirus, or a retrovirus carrying the constitutive mutant V449E (16) are also shown.

METHODS

Construction and expression of hβc receptor mutations.

We generated a deletion of two residues in CRD3 of $h\beta_c$ using the mutagenic oligonucleotide 5`GAGTGCTTCTTTGACGCAGTACTCAGCTGCTCC3` . A deletion of two residues was constructed in the E-F loop of CRD 3 using the oligonucleotide 5'CCCGTGCCCGACCCCCATGGCCAATACATC3'. Mutagenesis was performed using an $h\beta_c$ cDNA cloned into the mutagenesis vector pALTER and the procedure recommended by the manufacturer (Promega). Mutations were confirmed by DNA sequencing. For expression, the mutant receptor cDNA was excised from pALTER and cloned into the retroviral expression vector pRUFNeo (35). Retroviral DNA was used to transfect the ecotropic packaging cell line, Ψ2 {Mann}, and virus from G418 resistant cells was used to infect the murine haemopoietic cell lines, FDC-P1, BaF-B03 or CTL-EN by co-cultivation.

Cell lines, Growth Factors and flow cytometry.

Murine myeloid, IL-3/GM-CSF dependent FDC-P1 cells and derived cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 7.5% fetal bovine serum and murine GM-CSF (80U/ml; gift from Dr. G. Begley, Walter and Eliza Hall Institute for Medical Research, Melbourne Australia). Murine IL-3 dependent BaF-B03 cells were maintained in DMEM supplemented with 7.5% fetal bovine serum and murine IL-3 (300 U/ml; gift from Dr. A. Hapel, John Curtin School for Medical Research, Canberra, Australia). Murine IL-2 dependent CTL-EN cells (37 were maintained in RPMI supplemented with 10% bovine serum and murine IL-2 . BaF-B03 cells expressing high levels of the murine GM-CSF receptor α-subunit (mGMRα) and CTL-EN cells expressing high levels of the human IL-3 receptor α-subunit (hIL3Rα) have been reported previously (38).

After co-cultivation with producer cells FDC-P1, BaF-B03 or CTL-EN cells were harvested and selected in the presence of appropriate antibiotic and growth factor. Receptor expression was examined by flow cytometric analysis after staining with the FLAG-specific monoclonal antibody, M2 (Kodak/IBI). Briefly, cells were washed and resuspended in cold PBS supplemented with 5% BSA (PBSA). Cells were incubated with the M2 monoclonal antibody (1:300) for 20 minutes on ice, washed and subsequently incubated with biotinylated antimouse IgG (1:50, Vector Lab. Inc.) for 20 minutes on ice. After washing and resuspension in cold PBSA the cells were incubated with streptavidin conjugated phycoerythrin (1:50, Caltag Laboratories) for a further 20 minutes, washed, resuspended in PBSA + 0.01% sodium azide and analysed using an Epics-Profile II analyser (Coulter).

Mitogenic assays.

To assay proliferation, infected cells (FDC-P1, BaF-B03 and CTL-EN) were washed three times with PBS to remove growth factor and then assayed in triplicate (5000 cells per well) in a 96-well microtiter plate in the presence and absence of the appropriate growth factor. Cell growth was determined after 72 hours using a Cell Titer 96 non-radioactive cell proliferation assay (Promega). Quantitation was performed using an automated plate reader (BioRad).

RESULTS

Construction and expression of mutant hβc cDNA.

Our previous studies indicated that an interaction between the two membrane proximal domains (CRD3 and CRD4) of $h\beta_c$ may maintain the receptor in an inactive state (16, 24). To further define the sequences in CRD3 that are involved in this inhibitory interaction we used molecular modelling to identify regions predicted to lie in close proximity to the key ligand binding loops of CRD4. Molecular modelling suggested that the A-B and E-F loops of CRD3 may limit the mobility of the binding loops in CRD4 and therefore affect the conformational flexibility of this domain (see Figure 1). This idea is further supported by the interactions that occur between these loops in the inactive EPOR homodimer (25). Thus we targeted these loops for *in vitro* mutagenesis. In the cytokine receptor superfamily the A-B loop is of invariant size and lies between two highly conserved cysteine residues (39). Thus we can confidently predict the sequence of this loop in CRD3 (see Figure 2). The E-F loop is less well defined, however, there is a conserved cysteine in the E-strand which can be use for alignment and the F-strand has an alternating series of hydrophobic residues (see figure 6). To assess the function of the A-B loop we deleted two residues (ΔG254, A255) predicted to lie in the centre. The modified hβc cDNAs were cloned into the retroviral expression

vector pRUFNeo and introduced into the retroviral packaging cell line, Ψ2. A stable pool of transfected Ψ2 cells was produced by antibiotic selection. To test the ability of the ΔGA mutant to induce factor-independent growth, we infected the murine myeloid cell line, FDC-P1, and the IL-3 dependent pro-B cell line, BaF-B03 expressing mouse GM-CSF receptor α-subunit. We demonstrated surface expression of the mutant receptor on infected cells by flow cytometry following staining with the monoclonal antibody, M2 (data not shown).

Proliferation of infected FDC-P1 and BaF-B03 cells.

Uninfected FDC-P1 cells and G418-resistant FDC-P1 cells infected with pRUFNeo retrovirus carrying the wild type form of $h\beta_c$ grew in the presence of mGM-CSF but failed to proliferate after removal of growth factor (see Fig. 3). In contrast FDC-P1 cells infected with the ΔGA mutant gave rise to a population of FDC-P1 cells that proliferated in the absence of growth factor. These cells could be maintained in long term culture using complete medium lacking exogenous growth factor. The small degree of proliferation observed in the absence of factor following infection with the ΔGA retrovirus does not reflect the proportion of G418 resistant population that express receptor as a large proportion of cells are stained for $h\beta_c$ by flow cytometry (data not shown). Thus, it is possible that only a sub-population of FDC-P1 cells are capable of proliferation in response to this mutant. This implies that a threshold level of expression is required to support growth in the absence of factor or alternatively a critical signalling component is limiting in the majority of cells in the population. FDC-P1 cells infected with the DAT mutant did not consistently give rise to a population of cells that proliferated in the absence of growth factor (data not shown)

In common with wild type $h\beta_c$ and the I374N mutant (16) the ΔGA and ΔAT mutant were incapable of conferring IL-3 independent growth when used to infect the murine pro-B cell line BaF-B03 (Fig. 4A and 7A). However like many of the extracellular mutants both mutants conferred factor-independence when introduced into BaF-B03 cells expressing the mGMRα (Fig. 4B and Fig. 7B). In this case a sizeable sub-population of cells was capable of growth in the absence of growth factor implying that BaF3 cells contain all the required factors to support proliferation from these mutants. Importantly both mutants behave in a manner that is identical to the truncation mutant $h\beta_c$ ΔQP in which CRDs 1-3 are completely deleted (see ref 24)

To examine the ability of the ΔGA mutant to function in a ligand-dependent fashion we introduced ΔGA retrovirus into CTL-EN cells expressing hIL3Rα. We have shown

previously that activated forms of $h\beta_c$ do not confer growth factor-independent proliferation on these cells even in the presence of mGMRα. However, several of the $h\beta_c$ mutants confer a response to human IL-3 in CTL-EN cells expressing the human IL3Rα (38). These cells therefore provide a useful system for analysing the ability of mutant $h\beta_c$ to interact with another α-subunit and ligand. These cells therefore provide a useful system for analysing the ability of mutant $h\beta_c$ to interact with another α-subunit and ligand. We introduced the ΔGA mutant into these CTL-EN(hIL3Rα) cells and measured the proliferative response over a range of hIL-3 concentrations. As shown in Figure 5 the response to IL-3 is identical for cells expressing wild type $h\beta_c$ and the ΔGA mutant. Thus, the two residue deletion does not appear to perturb the ability of $h\beta_c$ to interact with hIL3Rα or to bind IL-3. We conclude therefore that this mutation does not cause a major disruption to the structure of $h\beta_c$. These results are also consistent with previous studies showing that the key ligand binding determinants in $h\beta_c$ are located in the B`-C` and F`-G` loops of CRD4 (20).

EXAMPLES

Example 1 - Methods for Detecting Antagonist or Agonist Molecules

Methods may include monitoring the growth of factor-dependent cell lines in the absence of growth factor and the presence of a candidate molecule (as described above). Alternative approaches include a biochemical assay to detect signalling, which assays might include any one of the following: tyrosine phosphorylation status of the receptor, the levels of phosphorylation on ERK1 and ERK2 MAP kinases, JAK and STAT tyrosine phosphorylation, STAT DNA binding activity, and activation of reporteer genes induced by specific signalling pathways.

Example 2 - Method for Producing Antibodies

The production of antibodies to CRD3 or an fragment thereof may be carried out by any of the techniques familiar to those skilled in the art. For example, production of monoclonal antibodies involves immunisation of animals with recombinant CRD3 or CRD3-derived peptide coupled to carrier protein. Derived antibodies are then screened for ability to detect recombinant CRD3 and hbc (and truncated derivatives) expressed on the surface of cultured animal or human cells. Antibodies are tested for function as described in Example 1 above.

Example 3 - Method of Screening for Small Molecule Antagonsits or Agonists

A diversity library such as a random combinatorial peptide or nonpeptide library is prepared according to any one or more of the following references. Alternatively a

library of natural products may be screened according to the methods known in the art and discussed herein.

Examples of chemically synthesized libraries are described in Fodor et al., 1991, Science 251:767-773; Houghten et al., 1991, Nature 354:84-86; Lam et al., 1991, Nature 354:82-84; Medynski, 1994, Bio/Technology 12:709-710; Gallop et al., 1994, J. Medicinal Chemistry 37(9):1233-1251; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten et al., 1992, Biotechniques 13:412; Jayawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, Science 249:386-390; Devlin et al., 1990, Science, 249:404-406; Christian, R. B., et al., 1992, J. Mol. Biol. 227:711-718); Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay et al., 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/0505 dated Apr. 18, 1991; and Mattheakis *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh *et al.* (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

The library can then be screened against CRD3 or fragment thereof using any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci.

USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and PCT Publication No. WO 94/18318.

Thus, the screening can be carried out by contacting the library members with CRD3 or fragment thereof immobilized on a solid phase and harvesting those library members that bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited hereinabove.

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CLAIMS

1. An agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding haemopoietic growth factor receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property.

2. An agonist or antagonist according to Claim 1 having agonist or antagonist properties to a member of the cytokine receptor family where the member is selected from any one of a group acting as a receptor for any one or more of the cytokines selected from the list including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, granulocyte-macrophage colony stimulating factor (GM-CSF), growth hormone (GH), prolactin (PRL), granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), thrombopoietin (TPO), leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), the p40 subunit of IL-12, leptin and newly discovered members of the cytokine receptor family.

3. An agonist or antagonist according to Claim 1 having agonist or antagonist properties to any one of GM-CSF, IL-5 and IL-3.

4. An agonist or antagonist according to either of Claim 2 or Claim 3 wherein the antagonist or agonist binds to a region selected from a group comprising the A-B loop and the E-F loop of CRD 3 or analogous domains within the family of haemopoietic growth factor receptors.

5. An agonist or antagonist according to Claim 4 wherein the antagonist or agonist binds to the A-B loop of CRD 3 or an analogous domain within the family of haemopoietic growth factor receptors.

6. An agonist or antagonist according to Claim 5 wherein the antagonist or agonist binds any one or more of the sequences listed in Figure 2.

7. An agonist or antagonist according to Claim 6 wherein the antagonist or agonist interferes with interaction of the A-B loop of CRD3 or an analogous region within the family of haemopoietic growth factors with the F'-G' loop of CRD4 or an analogous domain within the family of cytokine receptors.

8. An agonist or antagonist according to Claim 6 wherein the antagonist or agonist interferes with the interaction of the A-B loop of CRD3 or an analogous region within the family of cytokine receptors, with the B'-C' loop of CRD4 or an analogous domain within the family of cytokine receptors.

9. An agonist or antagonist according to Claim 4 wherein the antagonist or agonist binds to the E-F loop of CRD3 or an analogous domain within the family of haemopoietic growth factors.

10. An agonist or antagonist according to Claim 9 wherein the antagonist or agonist binds any one or more of the sequences listed in Figure 6.

11. An agonist or antagonist according to Claim 10 wherein the antagonist or agonist interferes with the interaction of the E-F loop of CRD3, or an analogous domain within the family of cytokine receptors with the F'G' loop of CRD4 or an analogous region within the family of cytokine receptors.

12. An agonist or antagonist according to Claim 10 wherein the antagonist or agonist interferes with the interaction of the E-F loop of CRD3, or an analogous region within the family of cytokine receptors with the B'C' loop of CRD4 or an analogous domain within the family of cytokine receptors.

13. An agonist or antagonist according to Claim 2 wherein the agonist or antagonist is selected from any one of a number of classes of compounds including antibodies, fragments of antibodies, peptides, oligosaccharides, oligonucleotides, or other organic or inorganic compounds.

14. A method for isolating an agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding cytokine receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property, said method including the steps of contacting candidate agonists or antagonists with CRD3 or fragments thereof, assaying candidate agents for their capacity to bind CRD3 or fragment thereof and testing for agonist or antagonist properties.

15. The method of claim 14 wherein the method includes fixing CRD3 or a fragment thereof to a substrate, contacting the fixed CRD3 or fragment with candidate antagonists or agonists, washing away material not bound to the fixed CRD3 or fragment, identifying the bound candidate antagonists or agonists, and testing the bound candidate antagonists or agonists for antagonist or agonist properties.

16. A pharmaceutical composition, said composition including

an agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding haemopoietic growth factor receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property, and

a suitable excipient.

17. A pharmaceutical composition according to Claim 16 wherein the agonist or antagonist has agonist or antagonist properties to a member of the cytokine receptor family where the member is selected from any one of a group acting as a receptor for any one or more of the cytokines selected from the list including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, granulocyte-macrophage colony stimulating factor (GM-CSF), growth hormone (GH), prolactin (PRL), granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), thrombopoietin (TPO), leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), the p40 subunit of IL-12, leptin and newly discovered members of the cytokine receptor family.

18. A pharmaceutical composition according to Claim 17 wherein the agonist or antagonist has agonist or antagonist properties to any one of GM-CSF, IL-5 and IL-3.

19. A pharmaceutical composition according to Claim 17 or Claim 18 wherein the agonist or antagonist binds to a region selected from a group comprising the A-B loop and the E-F loop of CRD 3 or analogous domains within the family of haemopoietic growth factor receptors.

20. A pharmaceutical composition according to Claim 19 wherein the agonist or antagonist binds to the A-B loop of CRD 3 or an analogous domain within the family of haemopoietic growth factor receptors.

21. A pharmaceutical composition according to Claim 20 wherein the agonist or antagonist binds any one or more of the sequences listed in Figure 2.

22. A pharmaceutical composition according to Claim 19 wherein the agonist or antagonist binds to the E-F loop of CRD3 or an analogous domain within the family of haemopoietic growth factor receptors.

23. A pharmaceutical composition according to Claim 22 wherein the agonist or antagonist binds any one or more of the sequences listed in Figure 6.

24. A pharmaceutical composition according to Claim 17 wherein the agonist or antagonist is selected from any one of a number of classes of compounds including antibodies, fragments of antibodies, peptides, oligosaccharides, oligonucleotides, or other organic or inorganic compounds.

25. A method of treating a condition in a human or animal by administering an agonist or antagonist of an haemopoietic growth factor, said agonist or antagonist capable of binding a region of the CRD3 of $h\beta_c$ or analogous domain of a corresponding haemopoietic growth factor receptor to thereby impact on an interaction between CRD3 and CRD4 or analogous domains to thereby effect an agonist or antagonist property, and

in a pharmaceutically acceptable form, in a suitable carrier and in a therapeutically effective dose..

26. A method of treating a condition in a human or animal according to Claim 25 wherein the agonist or antagonist has agonist or antagonist properties to a member of the cytokine receptor family where the member is selected from any one of a group acting as a receptor for any one or more of the cytokines selected from the list including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, granulocyte-macrophage colony stimulating factor (GM-CSF), growth hormone (GH), prolactin (PRL), granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), thrombopoietin (TPO), leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), the p40 subunit of IL-12, leptin and newly discovered members of the cytokine receptor family.

27. A method of treating a condition in a human or animal according to Claim 26 wherein the agonist or antagonist has agonist or antagonist properties to any one of GM-CSF, IL-5 and IL-3.

28. A method of treating a condition in a human or animal according to Claim 27 wherein the agonist or antagonist binds to a region selected from a group comprising the A-B loop and the E-F loop of CRD 3 or analogous domains within the family of haemopoietic growth factor receptors.

29. A method of treating a condition in a human or animal according to Claim 28 wherein the agonist or antagonist binds to the A-B loop of CRD 3 or an analogous domain within the family of haemopoietic growth factor receptors.

30. A method of treating a condition in a human or animal according to Claim 29 wherein the agonist or antagonist binds any one or more of the sequences listed in Figure 2.

31. A method of treating a condition in a human or animal according to Claim 28 wherein the agonist or antagonist binds to the E-F loop of CRD3 or an analogous domain within the family of haemopoietic growth factor receptors.

32. A method of treating a condition in a human or animal according to Claim 31 wherein the agonist or antagonist binds any one or more of the sequences listed in Figure 6.

33. A method of treating a condition in a human or animal according to Claim 26 wherein the agonist or antagonist is selected from any one of a number of classes of compounds including antibodies, fragments of antibodies, peptides, oligosaccharides, oligonucleotides, or other organic or inorganic compounds.

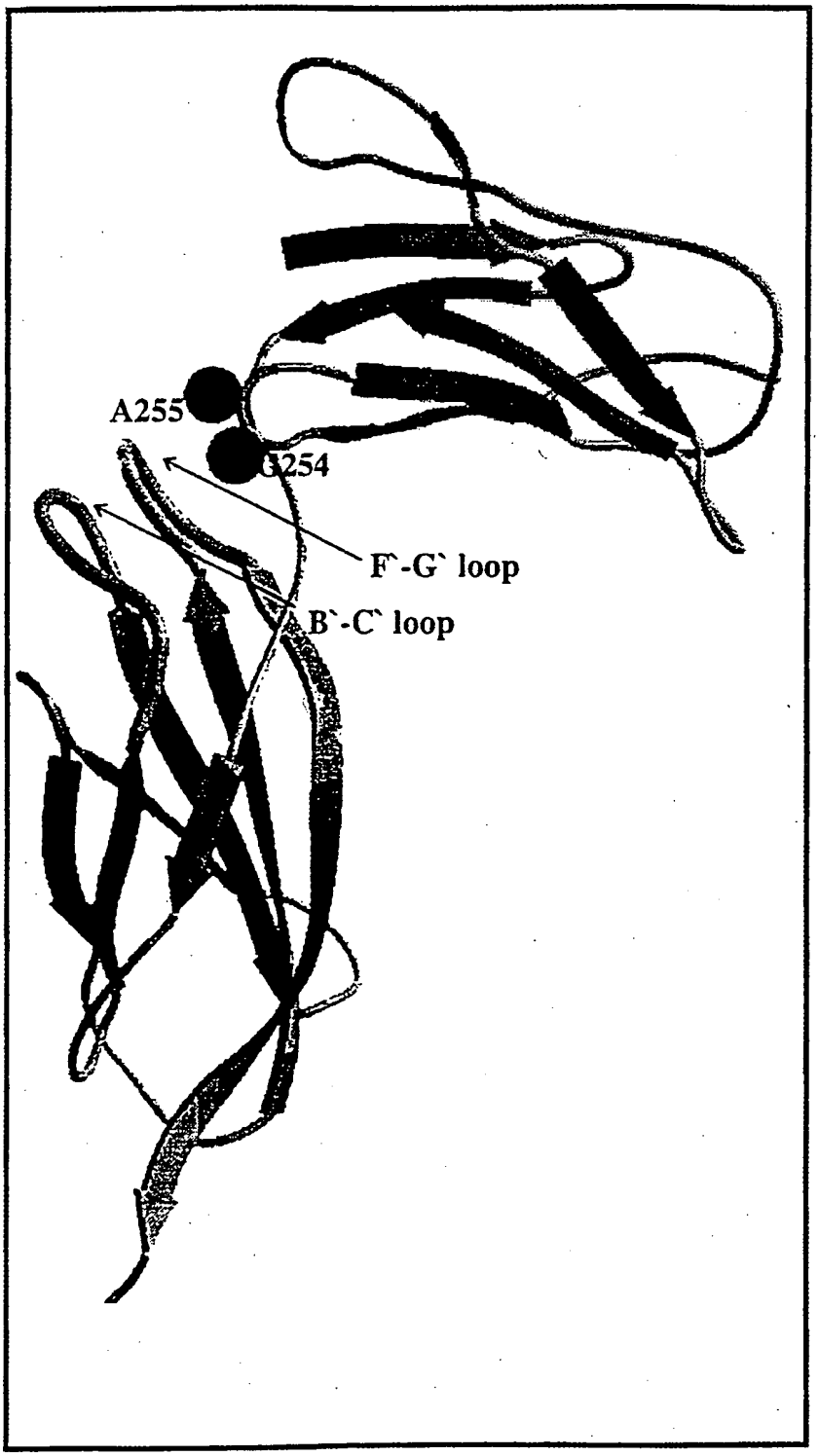


FIGURE 1

Cytokine-receptor subunits A-B loop alignment

hβc	CRM2	CFFDGA-AVLSCSW
mβc	CRM2	CFFDGI-QSLHCSW
mβIL3	CRM2	CFFDGI-QSLHCSW
hβc	CRM1	CYNDYT-SHITCRW
mβc	CRM1	CYNDYT-NHIICSW
mβIL3	CRM1	CYNDYT-NRIICSW
hTPOR	CRM2	CFTLDL-KNVTCQW
mTPOR	CRM2	CFTLDL-KMVTCQW
hTPOR	CRM1	CFSRTFED-LTCFW
mTPOR	CRM1	CFSQTFED-LTCFW
hEPOR		CFTERLED-LVCFW
hLIFR	CRM2	CETHDKKE-IICSW
mLIFR	CRM2	CETHDLKE-IICSW
hOBR	CRM2	CETDGYLTKMTCRW
mOBR	CRM2	CETDGYLTKMTCRW
hgp130		CIVNEGK-KMRCEW
mgp130		CIVNEGK-NMLCQW
h-gamma		CFVFNVEY-MNCIW
m-gamma		CFVFNIEY-MNCIW
hIL2Rb		CFY-NSRANISCVW
mIL2Rb		CFY-NSRANVSCMW
hGMRα		CFIYNAD-LMNCIW
hIL3Rα		CWIHDVD-FLSCSW
hGHR		CRSPERETF-SCHW

FIGURE 2

FIGURE 3

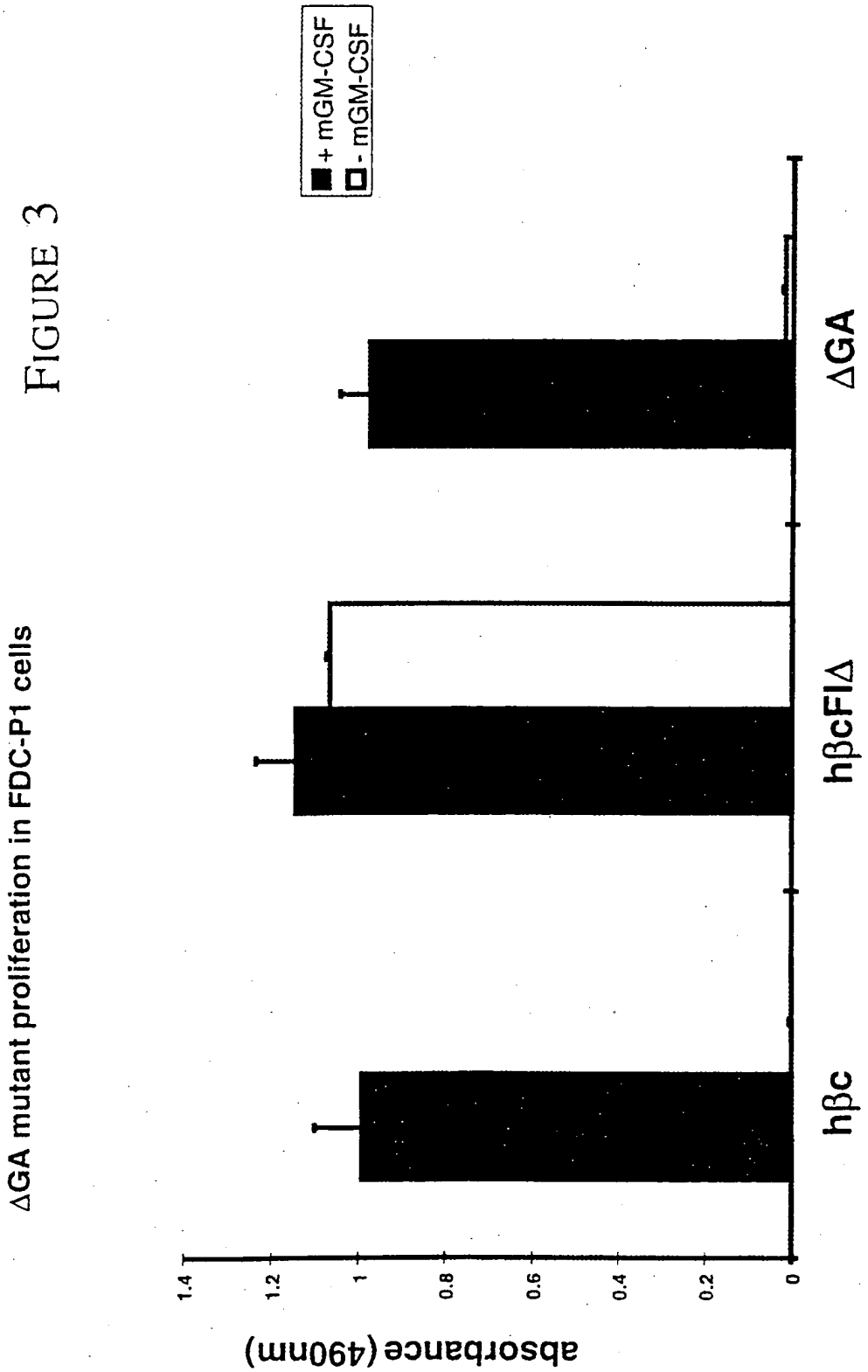

ΔGA mutant proliferation in FDC-P1 cells
+ mGM-CSF
- mGM-CSF
absorbance (490nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
hβc
hβcFIΔ
ΔGA

FIGURE 4A

ΔGA mutant proliferation in BaF-B03 cells

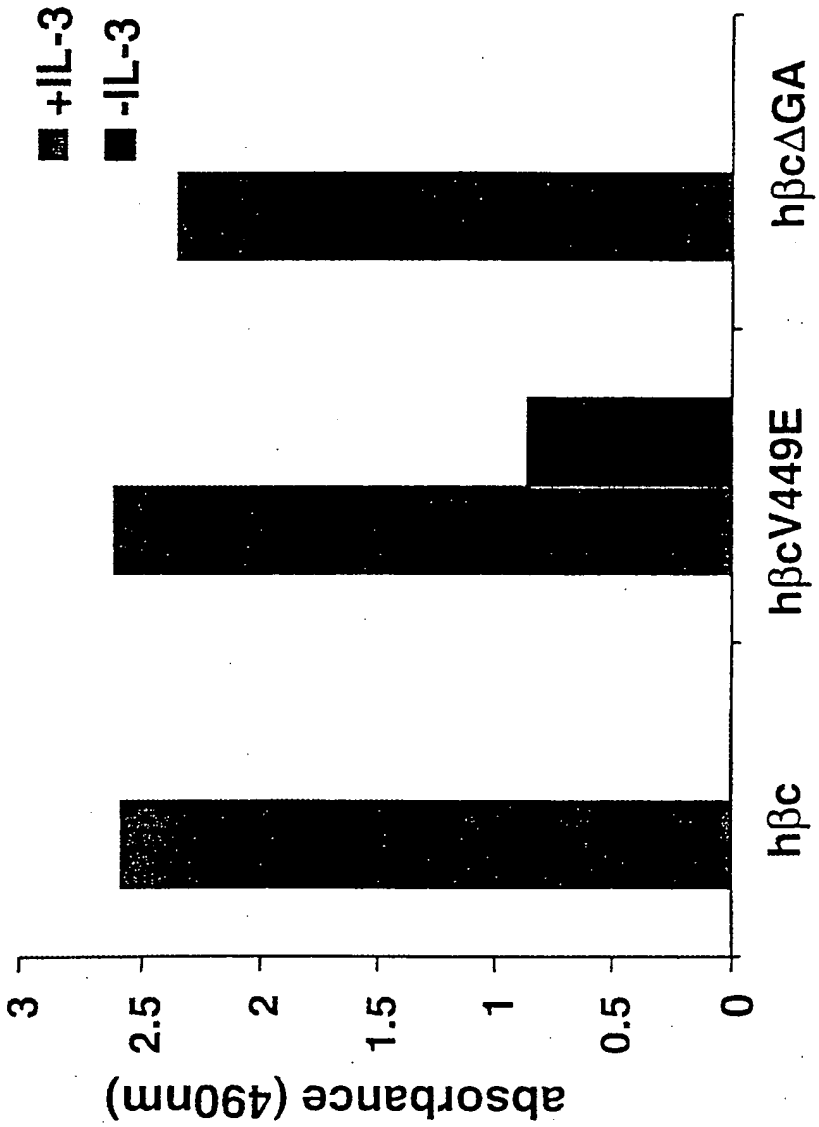


FIGURE 4B

ΔGA mutant proliferation in BaF-B03 cells expressing mGMRα

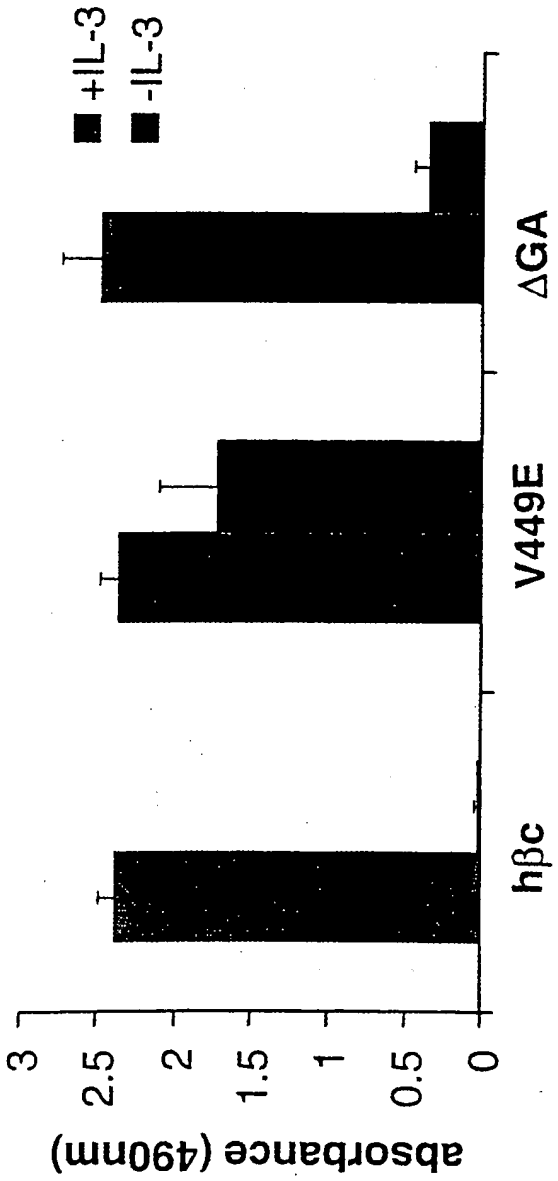


FIGURE 5

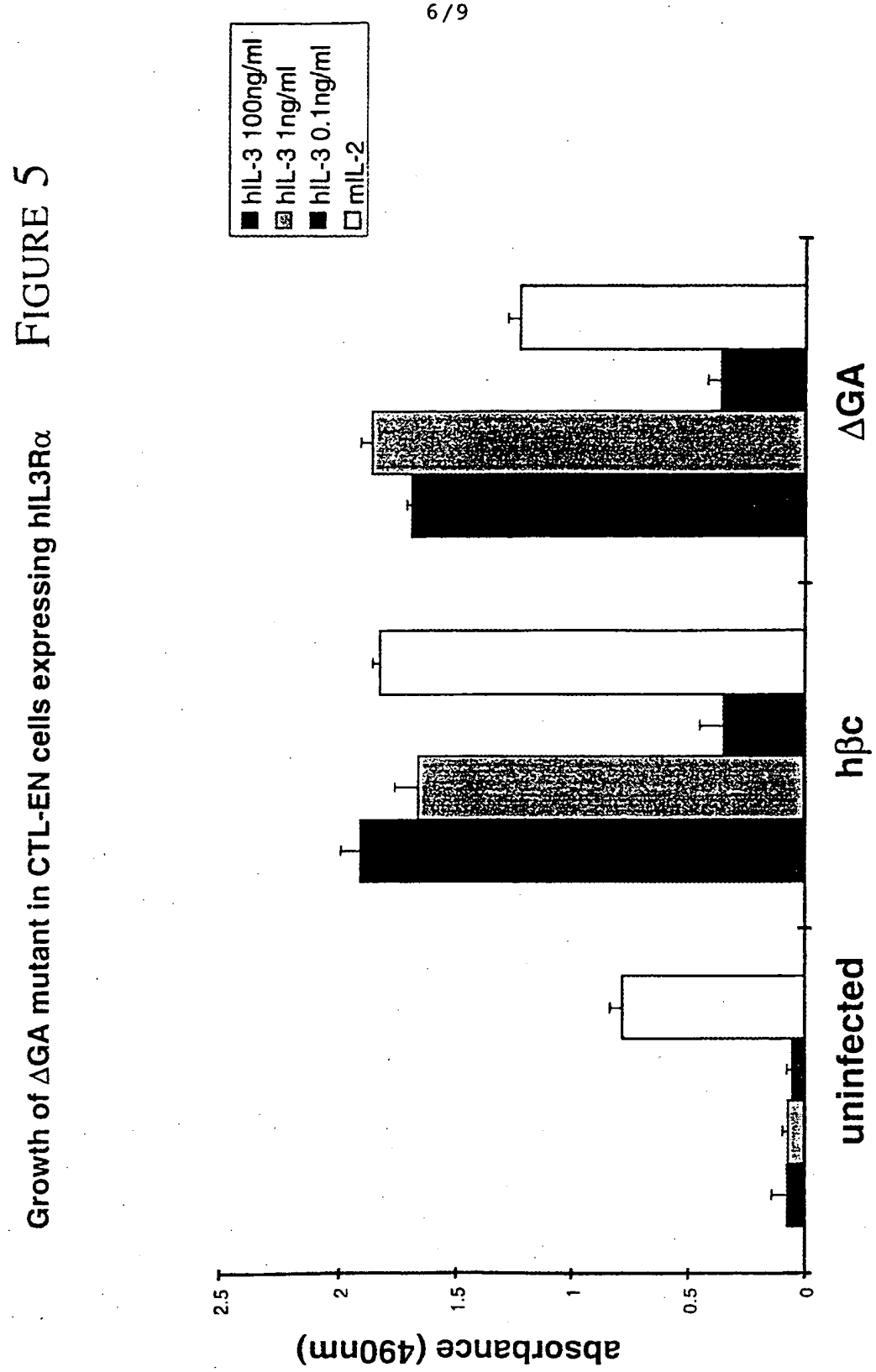

Growth of ΔGA mutant in CTL-EN cells expressing hIL3Rα
hIL-3 100ng/ml
hIL-3 1ng/ml
hIL-3 0.1ng/ml
mIL-2
absorbance (490nm)
2.5
2
1.5
1
0.5
0
uninfected
hβc
ΔGA

FIGURE 6

hβc	H	T	R	H	H	C	Q	I	P	V	P	D	P	A	T	H	G		Q	Y	I	V	S	V	Q	P
ghr	A	G	E	N	S	C	Y	F	N	S	S	F	T	S	I	W		I	P	Y	C	I	K	L	T	S
gp130	D	T	P	T	S	C	T	V	D	Y	S	T	V	Y			F	V	N	I	E	V	W	V	E	A
EPOR	A	V	R	F	W	C	S	L	P	T	A	D		T	S	S	F	V	P	L	E	L	R	V	T	A
TPOR CRM1	G	T	R	Y	V	C	Q	F	P	D	Q	E	E	V	R	L	F	F	P	L	H	L	W	V	K	N
TPOR CRM2	P	Q	F	S	R	C	H	F	K	S	R	N	D	S	I	I	H	I	L	V	E	V	T	T	A	P
OBR CRM2	D	G	F	Y	E	C	I	F	Q	P	I	F	L	L	S		G	Y	T	M	W	I	R	I	N	H
strand	e	e	e	e	E	E	E	e	e										e	E	E	E	E	E	E	E

FIGURE 7A

ΔAT proliferation in BaF-B03 cells

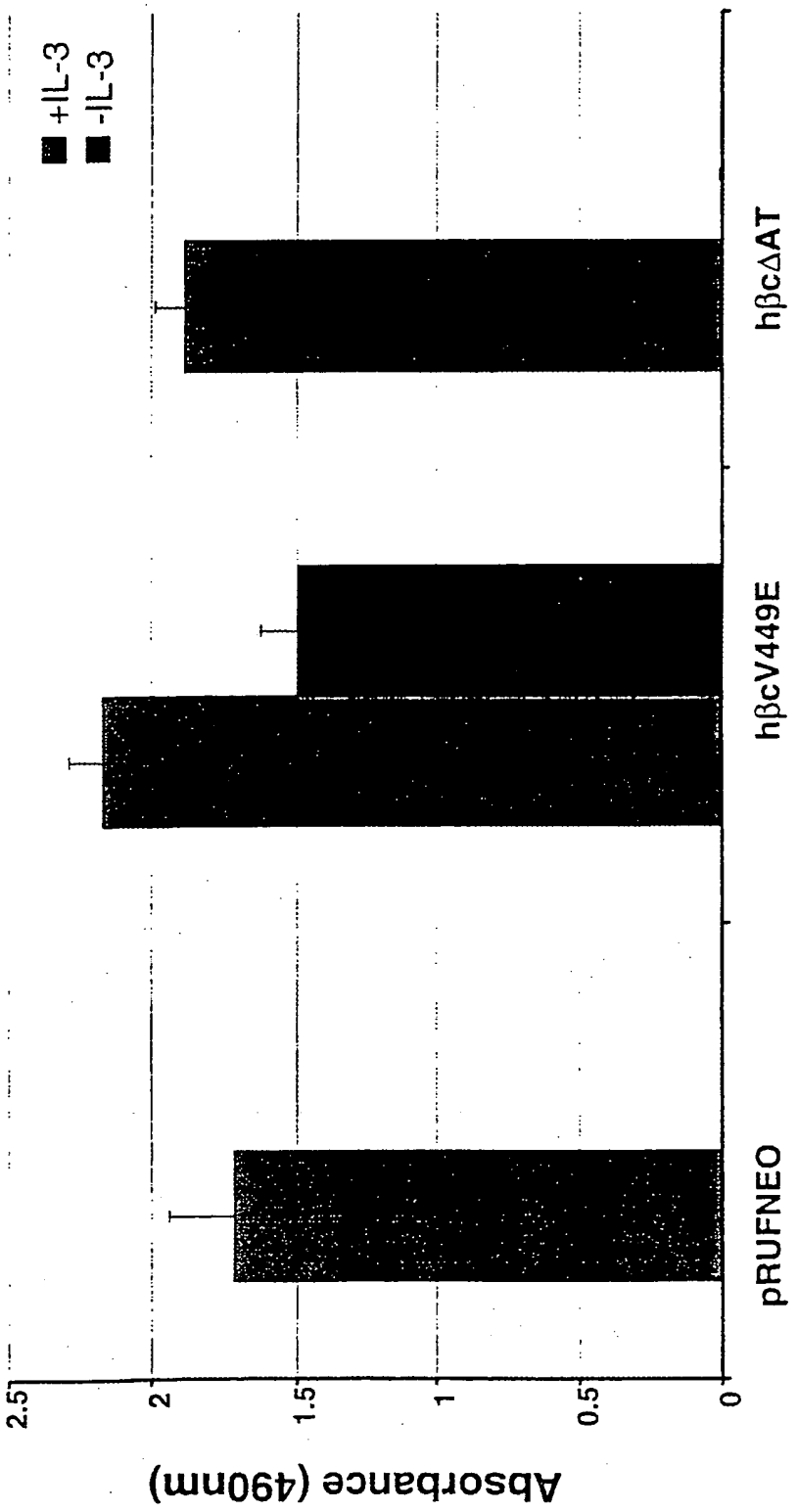
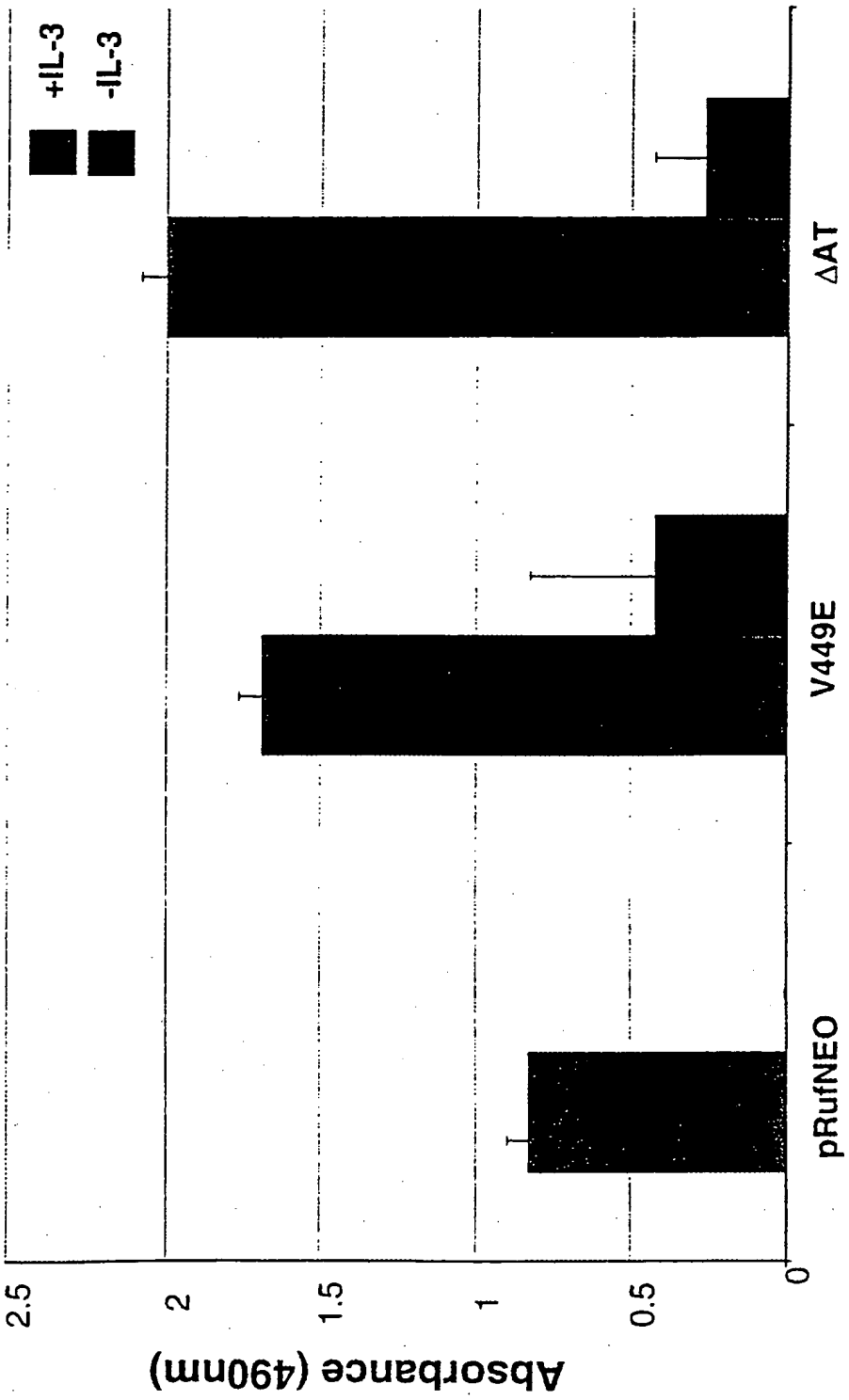


FIGURE 7B

ΔAT proliferation in BaF-B03 cells expressing mGMRα



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00394

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 16/24; C12N 15/24, 15/27; A61K 39/395, 38/20; A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
ELECTRONIC DATABASES

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: CA, Medline, Biosis, WPIDS - keywords

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9707215 (MEDVET SCIENCE) 27 February 1997 See whole document.	1-33
X	Blood Vol 89 Jan 1997 pages 355-369	1-33
Y	Gonda TJ et al, "Activating Mutations in Cytokine Receptors: Implications for Receptor Function and Role in Disease" See whole document especially page 359 column 2, page 360 column 2, page 362	1-13, 16-33

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2000	13 JUL 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer O.L. CHAI Telephone No : (02) 6283 2482

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00394

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Blood Vol 87 April 1996, pages 2641-2648 D'Andrea RJ et al, "Extracellular Truncations of hβc, the Common Signaling Subunit for Interleukin-3 (IL-3), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), and IL-5, Lead to Ligand-Independent Activation" See whole document especially page 2644 and 2646	1-33
X	Blood Vol 89 March 1997 pages 1471-1482 Bagley, CJ et al, "The Structural and Functional Basis of Cytokine Receptor Activation: Lessons from the Common β Subunit of the Granulocyte-Macrophage Colony-Stimulating Factor, and Interleukin-3 (IL-3), and IL-5 Receptors" See whole document especially pages 1474-1475.	1-33
Y	US 5516512 (GIST-BROCADES NV) 14 May 1996 See whole document.	1-13, 16-33
Y	WO 8702990 (SCHERING-BIOTECH) 21 May 1987 See whole document.	1-13, 16-33
Y	AU 73414/94 (MEDVET SCIENCE) 28 February 1995 See whole document.	1-13, 16-33
A	Blood Vol 83 May 1994 pages 2802-2808 D'Andrea R et al, "A Mutation of the Common Receptor Subunit for Interleukin-3 (IL-3), Granulocyte-Macrophage Colony-Stimulating Factor, and Interleukin-3 (IL-3), and IL-5 that Leads to Ligand Independence and Tumorigenicity See whole document.	1-33
A	Blood Vol 82 October 1993, pages 1960-1974 Miyajima A et al, "Receptors for the Granulocyte-Macrophage Colony-Stimulating Factor, and Interleukin-3, and Interleukin-5" See whole document.	1-33

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00394

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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		SG	52238				

END OF ANNEX